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Super Dosing of IP6 Phytase in Phosphorus-Deficit Diets to Replace Added Phosphorus and Evaluate Growth Performance, Nutrient Digestibility and Bone Mineralization of Broiler Chickens

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Phosphorus in phytate is poorly available to monogastric animals due to their lack of the phytase enzyme, leading to increased phosphorus excretion and environmental pollution. Additionally, broiler diets high in phytate and low in available phosphorus negatively affect growth performance, nutrient absorption, and bone mineralization. This study was planned to evaluate the effect of adding IP6 phytase as a dietary replacement of added phosphorus with generic phosphorus in broiler diets on growth performance, nutrient digestibility, and bone mineralization. Seven hundred and fourteen (714) day-old Ross-308 broiler chicks were randomly distributed into forty-two experimental pens (6 replicates and 17 birds/pen). Seven isonitrogenous experimental diets were formulated for starter (1-21 days) and finisher (22-28 days) phases. The first diet was served as control (C), formulated following nutrient recommendations for Ross® broilers. Three negative control diets were formulated with available phosphorus levels of 0.15%, 0.18%, and 0.21%, each supplemented with phytase at 1000 FTU/kg and 2000 FTU/kg across both phases. Phytase supplementation increased the body weight gain during days 01-07 ($p=0.01$), 08-14 ($p=0.004$), 15-21 ($p=0.0001$), 22-28 ($p=0.036$) and 0-28 ($p<0.001$). Feed intake was not significantly affected during days 01-07 ($p=0.114$) and 08-14 (0.094). However, higher levels of phytase (2000 FTU/kg with 0.21% Av. P) increased feed intake during days 15-21 (0.037) while a decrease in feed intake was observed during days 22-28 (0.009). Increased level of phytase (2000 FTU/kg) also improved the digestibility of dry matter ($p=0.049$), crude protein ($p=0.03$), and ether extract ($p=0.026$). Further, bone mineralization improved with the addition of phytase in the broiler diet. In conclusion, phytase supplementation can replace added phosphorus by releasing bound phosphorus from phytate without compromising the growth performance, nutrient digestibility, and tibia mineral content in broilers.
Keywords: Phytase, anti-nutritional factors, environmental pollution, poultry, growth performance, bone health.

INTRODUCTION

Grains are major components of poultry feed worldwide and contain inorganic phosphorus (P) in the form of phytate. The availability of phosphorus present in phytate is very low for monogastric animals due to the deficiency of phytase enzyme (Dersjant-Li and Awati, 2015.). Consequently, animals fed a diet high in phytate excrete more phosphorus through their feces, contributing to environmental pollution (Lim *et al.*, 2024). Additionally, the presence of phytate in poultry feed has adverse effects on broiler performance and bone mineralization (Nuamah *et al.*, 2024). Several studies have shown that broilers fed a diet low in phosphorus and high in phytic acid result in decreased weight gain, poor efficiency, low digestion and nutrient absorption, and lower mineral deposition in bones (Jiang *et al*., 2013; Walk and Rao, 2020). Phytate acts as an anti-nutritional factor for poultry due to its chelating properties with calcium (Ca), zinc, copper, magnesium, iron, and potassium (Humer *et al*., 2015; Venter *et al*., 2024). Calcium and phosphorus are essential nutrients involved in many biological processes, with 99% of Ca and 80% of P stored in the skeleton as hydroxyapatite,

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contributing significantly to bone development and mineralization (Proszkowiec-Weglarz and Angel, 2013). Ca plays critical roles in metabolism, enzyme activation, blood coagulation, neuromuscular activity, and bone health, while P is crucial for phospholipid formation, nucleotide synthesis, and energy metabolism (Veum, 2010). The presence of phytate in the broiler diet increases the endogenous sodium losses resulting in sodium deficiency and reduces the efficiency of sodium-potassium ATPases present in the gastrointestinal tract, which are necessary for proper digestion, and absorption of nutrients (Woyengo and Nyachoti, 2013). To overcome the limitations posed by phytate, inorganic phosphorus sources like mono-calcium phosphate are often added to poultry diets to meet the birds' phosphorus needs. However, these sources are expensive, and excess P in the diet can increase feed costs and contribute to environmental pollution (Cambra-López *et al*., 2021). In contrast, dietary P deficiency in diet leads to production losses and poor bone mineralization, which can also be viewed as a welfare issue(Valable *et al*., 2018).

Phytase, an exogenous enzyme, has been introduced to poultry diets to break down phytate, releasing bound phosphorus and improving its availability to birds. Supplementing broiler diets with microbial phytase not only enhances phosphorus availability but also increases the digestibility of calcium, amino acids, and other minerals, leading to better growth performance and improved bone health(Butani and Parnerkar, 2015; Gulizia *et al*., 2022). The use of phytase minimizes nutrient excretion in the feces by improving their availability and absorption in the small intestine, all while lowering feed costs and maintaining broiler growth performance (Valente Junior *et al*., 2024). A high dose of phytase in the diet can lead to nearly complete phytate breakdown and further reduce the anti-nutritive effects of phytate in diets high in phytate phosphorus(Broch *et al*., 2018; Baradaran *et al*., 2021). Walters *et al*. (2019) state that high phytase doses (2,000FTU/kg) optimize bird performance, nutrient digestibility, and bone mineralization. Baradaran *et al*. (2021) found that adding phytase (500FTU/kg) to low-phosphorus diets helps recover growth performance and bone mineralization lost due to phosphorus deficiency. Phytase supplementation (1000 FTU/kg) reduces phosphorus needs, enhances nutrient digestibility, and improves broiler growth and tibia calcium and phosphorus contents, saving up to 9.17% in costs (Iqbal *et al*., 2023).

We hypothesize that phytase supplementation can release phytic phosphorus from low-phosphorus diets, thereby reducing the need for additional inorganic phosphorus. Therefore, this study was planned to evaluate the effects of adding IP6 phytase in phosphorus-deficient diets as a replacement for added phosphorus, with a focus on growth performance, nutrient digestibility, and bone mineralization in broiler chickens.

MATERIALS AND METHODS

The 28-day trial was conducted at the Dr. Raja Muhammad Akram Animal Nutrition Center, Institute of Animal and Dairy Sciences, University of Agriculture Faisalabad, Pakistan, following approval from the Graduate Studies and Research Board (GSRB).

***Housing and Animals*:** Seven hundred fourteen (714) day-old Ross-308 broiler chicks were randomly assigned to 7 treatment groups, with 6 replicates per treatment and 17 chicks per cage. The chicks were purchased from a local hatchery. Upon arrival, they were checked for physical health and weighed using a digital scale. A sugar solution (250 g/L) was provided to alleviate transportation stress. The chicks were housed in separate pens within an experimentally controlled environment. Birds in each treatment group were fed experimental diets *ad libitum* throughout the trial.

***Experimental diets*:** Seven iso-nitrogenous (22% CP) experimental diets were formulated for the starter (1-21 days), and finisher (22-28 days) phases (Tables 1 and 2, respectively). The nutrient composition for each phase is provided in the corresponding tables. The first diet served as the control (C) and was formulated according to nutrient recommendations for Ross® broilers. Three negative control diets were formulated using a phosphorus matrix of 0.15%, 0.18%, and 0.21% available phosphorus, and each diet supplemented with phytase at 1000 FTU/kg and 2000 FTU/kg.

Winzyme HTR® is a thermostable 6-Phytase with the concentration of 20000 FTU/g supplied by Suntaq International Limited, China

Nutrimin® A mineral mixture; it contains Iron 10000 mg, Copper 12000 mg, Selenium 360 mg, Manganese 140000 mg, Cobalt 400 mg, Iodine 1800 mg, and Zinc 120000 mg

Vitamin premix HD501® A vitamin mixture; it contains Vitamin A 20000 KIU, Vitamin D_3 5000 KIU, Vitamin E 48000 mg, Vitamin K_3 3500 mg, Vitamin B_1 3000 mg, Vitamin B_2 8800 mg, Vitamin B_6 6800 mg, Vitamin B_{12} 20000 mg, Folic acid 1600 mg, Niacin 60000 mg, Calcium D-pantothenate 12000mg.

Data collection and measurements

***Feed intake and composition*:** The weekly feed intake for each replicate was determined by subtracting the feed refusals from the total feed provided to the birds during that week. Feed samples were collected and analyzed for dry matter (DM), crude protein (CP), ether extract (EE), and ash content using standard laboratory techniques as outlined by the Association of Official Analytical Chemists (AOAC) (Horwitz and Latimer, 2000), with modifications as discussed in Xia *et al*. (2018).

***Growth performance*:** Birds were weighed on days 1, 7, 14, 21, and 28 using a weighing balance, and body weight gain (BWG) was calculated. The feed conversion ratio (FCR) was determined based on feed intake and BWG. Mortality and the

weights of deceased birds were recorded throughout the trial to calculate corrected FCR and feed intake.

***Nutrient digestibility (%)*:** A digestibility trial was conducted on the 27th day of the experimental period. For this trial, Celite® (a source of acid-insoluble ash) was added to the feed at 1% from days 21 to 27. Plastic sheets were placed under each pen to collect droppings, which were composited replicate-wise and stored at -20°C until nutrient digestibility analysis (Horwitz and Latimer, 2000). Digestibility was then calculated using the following equation:

Nutrient digestibility %

$$= 100\text{-}100\times\left(\frac{\%\text{ marker in feed}}{\%\text{ marker in feces}} \times \frac{\%\text{ nutrient in feces}}{\%\text{ nutrient in feed}}\right)$$

***Bone mineralization (%)*:** Bone mineralization was measured by slaughtering two birds from each pen. From each bird, the right tibia was removed, cleaned, and stored in a freezer for later analysis of tibia ash. The tibia samples were dried in an oven at 105°C for 48 hours, and fat was removed using a Soxhlet apparatus with petroleum ether for 16 hours. After fat extraction, the tibia were dried further, then detached from the filter paper, weighed, and placed in a muffle furnace at 600°C for 18 hours. The remaining residue was weighed to calculate ash content (Lalpanmawia *et al.*, 2014; Boney and Moritz, 2017). The formula for calculating tibia ash percentage is as follows:

Table 1. Ingredient composition of starter diet.

Treatments	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Ingredients	Control	Phytase 1000 FTU/kg			Phytase 2000 FTU/kg		
		0.15% Av. P	0.18% Av. P	0.21% Av. P	0.15% Av. P	0.18% Av. P	0.21% Av. P
Corn	50.83	55.38	56.15	56.92	55.38	56.15	56.92
Rice polishing(s)	6	6	6	6	6	6	6
Soybean meal	28.93	27.21	26.93	26.66	27.21	26.93	26.66
Canola meal	5	5	5	5	5	5	5
Poultry by-product meal	3	3	3	3	3	3	3
Poultry fat	2.55	0.74	0.43	0.13	0.74	0.43	0.13
Calcium carbonate	1.22	1.05	1.02	1.00	1.05	1.02	1.00
Mono-Calcium phosphate	**1.10**	**0.42**	**0.30**	**0.18**	**0.42**	**0.30**	**0.18**
Sodium chloride	0.24	0.24	0.23	0.23	0.24	0.23	0.23
Sodium bicarbonate	0.23	0.10	0.06	0.02	0.10	0.06	0.02
Lysine sulphate,55%	0.34	0.37	0.37	0.37	0.37	0.37	0.37
DL-Methionine,99.5%	0.29	0.25	0.24	0.24	0.25	0.24	0.24
L-Threonine, 99%	0.05	0.03	0.03	0.03	0.03	0.03	0.03
L-Isoleucine	0.04	0.04	0.04	0.04	0.04	0.04	0.04
L-Valine	0.08	0.06	0.07	0.07	0.06	0.07	0.07
Vitamin premix, HD501®	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix, HD605 ®	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Winzyme HTR®	**-**	**0.005**	**0.005**	**0.005**	**0.01**	**0.01**	**0.01**
Total	100	100	100	100	100	100	100
Nutrient%							
DM	89.84	89.53	89.48	89.43	89.53	89.48	89.43
ME kcal/kg	3000	3000	3000	3000	3000	3000	3000
CP	22	22	22	22	22	22	22
EE	6.29	4.61	4.32	4.04	4.61	4.32	4.04
CF	3.29	3.29	3.29	3.29	3.29	3.29	3.29
Ca	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Av. P	0.45	0.30	0.27	0.24	0.30	0.27	0.24
Phytic P	0.27	0.28	0.28	0.28	0.28	0.28	0.28
Na	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Cl	0.23	0.23	0.23	0.23	0.23	0.23	0.23
DEB	228	206	201	196	206	201	196
Lysine, digestible	1.22	1.22	1.22	1.22	1.22	1.22	1.22
M+C, digestible	0.89	0.89	0.89	0.89	0.89	0.89	0.89
Thr, digestible	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Trp, digestible	0.23	0.24	0.25	0.22	0.24	0.25	0.22
Arg, digestible	1.35	1.33	1.32	1.32	1.33	1.32	1.32
Val, digestible	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Leu, digestible	1.62	1.61	1.61	1.61	1.61	1.61	1.61
Ile, digestible	0.84	0.84	0.84	0.84	0.84	0.84	0.84

Table 3. Ingredient composition of finisher diets (days 22-28).

Treatments	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Ingredients	Control	Phytase (1000 FTU/kg)			Phytase (2000 FTU/kg)		
		0.15% Av. P	0.18% Av. P	0.21% Av. P	0.15% Av. P	0.18% Av. P	0.21% Av. P
Corn	55.47	60.04	60.92	61.08	60.04	60.92	61.08
Rice polishing(s)	6	6	6	6	6	6	6
Soybean meal	22.18	20.43	20.13	20.9	20.43	20.13	20.9
Canola meal	6	6	6	6	6	6	6
Poultry by-product meal	3	3	3	2.33	3	3	2.33
Poultry fat	4.24	2.41	2.06	1.92	2.41	2.06	1.92
Calcium carbonate	1.06	0.90	0.87	0.87	0.90	0.87	0.87
Mono-Calcium phosphate	**0.90**	**0.22**	**0.06**	**-**	**0.22**	**0.06**	**-**
Sodium chloride	0.24	0.24	0.23	0.24	0.24	0.23	0.24
Sodium bicarbonate	0.24	0.1	0.06	0.03	0.1	0.06	0.03
Lysine sulphate, 55%	0.26	0.29	0.29	0.29	0.29	0.29	0.29
DL-Methionine, 99.5%	0.24	0.21	0.20	0.20	0.21	0.20	0.20
L-Threonine, 99%	0.04	0.02	0.02	0.01	0.02	0.02	0.01
L-Isoleucine	0.01	0.01	0.02	0.01	0.01	0.02	0.01
Vitamin premix, HD501®	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix, HD605®	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Winzyme HTR®	**-**	**0.005**	**0.005**	**0.005**	**0.005**	**0.005**	**0.005**
Total	100	100	100	100	100	100	100
Nutrients%							
DM	89.92	89.62	89.55	89.50	89.62	89.55	89.50
ME kcal/kg	3150	3150	3150	3150	3150	3150	3150
CP	19.5	19.5	19.5	19.5	19.5	19.5	19.5
EE	8.02	6.33	6.01	5.83	6.33	6.01	5.83
CF	3.15	3.15	3.15	3.18	3.15	3.15	3.18
Ca	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Av. P	0.39	0.24	0.21	0.18	0.24	0.21	0.18
Phytic P	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Na	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Cl	0.23	0.23	0.23	0.23	0.23	0.23	0.23
DEB	201	180	175	173	180	175	173
Lysine, digestible	1.03	1.03	1.03	1.03	1.03	1.03	1.03
M+C, digestible	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Thr, digestible	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Trp, digestible	0.20	0.22	0.22	0.22	0.22	0.22	0.22
Arg, digestible	1.18	1.15	1.15	1.15	1.15	1.15	1.15
Val, digestible	0.79	0.79	0.79	0.80	0.79	0.79	0.80
Leu, digestible	1.47	1.46	1.46	1.46	1.46	1.46	1.46
Ile, digestible	0.71	0.71	0.71	0.71	0.71	0.71	0.71

$$\text{Tibia ash \%} = \frac{\text{weight of tibia ash in gram}}{\text{weight of tibia in gram}} \times 100$$

The bone sample was used to determine the calcium and phosphorus percentages. Samples were prepared using wet digestion (AOAC, 2000), and the concentrations of phosphorus and calcium were measured photometrically using the Fluitest PHOS diagnostic kit (Analyticon Biotechnologies AG).

***Statistical analysis*:** The collected data were analyzed using Analysis of Variance (ANOVA) in Minitab® under a Completely Randomized Design. Treatment means were compared using Tukey's test (Steel *et al*., 1997).

RESULTS

***Growth Performance*:** During days 1 to 7, feed intake (FI) was similar (P > 0.05) across all treatments. However, negative control diets supplemented with 2000 FTU/kg of phytase resulted in significantly better weight gain (WG) and improved feed conversion ratio (FCR) (P<0.05) compared to the control group. The highest weight gain (202.1 g/bird) and improved FCR (0.82) were observed in treatment T7 (0.21% available phosphorus with 2000 FTU/kg phytase) during the first week (Table 3.1).

During the second, third, and fourth weeks, negative control diets supplemented with 2000 FTU/kg of phytase resulted in significantly better (P < 0.05) BWG and FCR compared to the

other treatment groups. The T7 group (0.21% available phosphorus with 2000 FTU/kg phytase) showed highest WG during second (335.1 g/bird) with 1.17 FCR) and third week (580.9 g/bird with 1.26 FCR). However, T6 (0.18% available phosphorus with 2000 FTU/kg phytase) achieved highest WG (685.5 g/bird) during fourth week. FI during the second week showed a trend toward significance (P = 0.094), with maximum FI (405.4 g/bird) in T6 (0.18% available phosphorus with phytase at 2000 FTU/kg). During the third and fourth weeks, FI showed a significant difference (P < 0.05) among treatments, with the highest values observed in T7 (731.6 g/bird,) and in T6 (1098.5 g/bird), respectively (Table 3.1).

***Nutrient digestibility*:** Negative control diets supplemented with phytase showed significantly higher (P < 0.05) digestibility of crude protein (CP) and ether extract (EE) compared to the control group (Table 3.2). Additionally, there was a trend (P = 0.05) suggesting that super dosing phytase in low-phosphorus diets can enhance dry matter (DM) digestibility. Among the diets, the group supplemented with 2000 FTU/kg phytase (0.21% available phosphorus) demonstrated the highest DM digestibility (85.39%). Similarly, the T7 group (0.21% available phosphorus with 2000 FTU/kg phytase) showed the highest digestibility of CP (75.46%) and EE (86.37%).

Table 3.1. Effect of phytase supplementation on growth performance.

Days	Growth parameters	T1	T2	T3	T4	T5	T6	T7	P-value
			Phytase 1000 FTU/kg			Phytase 2000 FTU/kg			
		Control	0.15% Av.P	0.18% Av.P	0.21% Av.P	0.15% Av.P	0.18% Av.P	0.21% Av.P	
01-07	Weight gain (g)	186.1^{c}±0.8	189.0^{bc}±2.0	195.5^{abc+}±2.7	199.5^{ab}±3.0	198.3^{abc}±2.8	201.6^{a}±3.9	202.1^{a}±3.2	0.001
	Feed intake (g)	168.7^{a+}±2.6	163.2^{a}±1.7	166.9^{a}±2.3	170.9^{a}±3.5	165.9^{a}±2.0	172.8^{a}±1.9	165.9^{a+}±2.2	0.114
	FCR	0.91^{a}±0.01	0.86^{ab}±0.01	0.85^{b}±0.004	0.86^{b}±0.01	0.84^{b}±0.01	0.86^{b}±0.02	0.82^{b}±0.01	0.001
08-14	Weight gain (g)	291.7^{b}±4.4	304.3^{ab}±5.9	319.7^{ab}±10.8	316.9^{ab}±6.1	321.9^{ab}±6.3	334.1^{a}±11.5	335.1^{a+}±7.1	0.004
	Feed intake (g)	399.2^{a}±19.4	357.7^{a}±8.7	389.3^{a}±9.6	394.1^{a}±5.5	384.6^{a}±9.6	405.4^{a}±4.3	391.4^{a}±11.6	0.094
	FCR	1.36^{a}±0.06	1.17^{b}±0.03	1.22^{ab}±0.02	1.24^{ab}±0.02	1.19^{b}±0.02	1.22^{ab}±0.05	1.17^{b}±0.04	0.019
15-21	Weight gain (g)	512.6^{c}±3.9	528.6^{bc}±6.9	541.2^{bc}±7.5	536.4^{bc}±7.9	551.8^{ab}±6.0	546.3^{abc}±10.4	580.9^{a}±14.3	0.001
	Feed intake (g)	685.8^{b}±3.7	708.8^{ab}±6.9	701.9^{ab}±7.9	703.3^{ab}±3.6	712.8^{ab}±14.0	698.0^{ab}±13.0	731.6^{a}±6.9	0.037
	FCR	1.33^{a}±0.01	1.34^{a}±0.01	1.29^{ab}±0.01	1.31^{ab}±0.01	1.29^{ab}±0.02	1.27^{ab}±0.02	1.26^{b}±0.03	0.014
22-28	Weight gain (g)	626.3^{a}±8.9	625.0^{a}±19.1	634.0^{a}±10.5	631.0^{a}±17.1	635.7^{a}±17.9	685.5^{b}±7.57	668.3^{b}±16.7	0.036
	Feed intake (g)	1089.8^{a}±16	1051.5^{ab}±15	1050.0^{ab}±14	1045.2^{ab}±5	1029.7^{b}±11	1098.5^{a}±18	1052.7^{ab}±11	0.009
	FCR	1.74^{a}±0.03	1.68^{ab}±0.04	1.65^{ab}±0.02	1.66^{ab}±0.04	1.62^{ab}±0.03	1.60^{b}±0.02	1.57^{b}±0.03	0.009
0-28	Weight gain (g)	1616.8^{d}±11	1646.9^{cd}±19	1690.4^{bcd}±23	1683.5^{bcd}±2	1707.7^{abc}±22	1767.4^{ab}±12	1786.5^{a}±24	0.001
	Feed intake (g)	2343.5^{ab}±6	2281.3^{b}±24	2308.2^{ab}±24	2313.5^{ab}±12	2293.0^{ab}±23	2374.8^{a}±20	2341.6^{ab}±19	0.024
	FCR	1.44^{a}±0.01	1.38^{b}±0.01	1.36^{bc}±0.01	1.37^{b}±0.01	1.34^{bc}±0.01	1.34^{bc}±0.02	1.31^{c}±0.02	0.001

*Means with different superscripts amongst treatments differed significantly (P<0.05) in a row.

Table 3.2. Effect of phytase on nutrient digestibility.

Nutrient %	T1	T2	T3	T4	T5	T6	T7	*P*-value
	Experimental diets							
DM	84.91^{a}±0.6	82.26^{a}±0.8	80.90^{a}±1.0	83.63^{a}±1.0	84.73^{a}±0.9	84.40^{a}±1.0	85.39^{a}±0.7	0.049
CP	69.75^{b}±1.0	71.47^{ab}±0.8	71.62^{ab}±0.8	72.28^{ab+}±1.0	71.99$^{ab=}$±1.0	72.71$^{ab=}$±0.9	75.46^{a}±0.7	0.030
EE	77.49^{b}±0.9	80.09^{ab}±1.4	83.36^{ab}±2.0	84.53^{ab}±1.0	85.11^{ab}±2.0	84.95^{ab}±2.0	86.37^{a}±1.0	0.020

*Means with different superscripts amongst treatments differed significantly (P<0.05) in a row.

T1= Control, T2= 0.15% Available Phosphorus with *Phytase* @ 1000 FTU/kg, T3= 0.18% Available Phosphorus with *Phytase* @ 1000 FTU/kg, T4= 0.21% Available Phosphorus with *Phytase* @ 1000 FTU/kg, T5= 0.15% Available Phosphorus with *Phytase* @ 2000 FTU/kg, T6= 0.18% Available Phosphorus with *Phytase* @ 2000 FTU/kg, T7= 0.21% Available Phosphorus with *Phytase* @ 2000 FTU/kg

Table 3.3. Effect of phytase supplementation on bone mineralization.

Mineral %	T1	T2	T3	T4	T5	T6	T7	*P*-value
Tibia ash	42.5^{c}±0.3	43.4^{bc}±0.8	44.4^{bc}±1.4	44.97^{abc}±0.8	46.3^{abc}±1.3	47.5$^{ab=}$±0.3	48.8^{a}±0.3	0.019
Ca	14.4^{c}±0.4	15.2^{bc}±0.4	16.16^{abc}±0.5	16.89^{ab}±0.3	16.49^{abc}±0.8	17.2^{ab}±0.4	17.9^{a}±0.3	0.014
P	6.8^{a}±0.2	7.65^{a}±0.5	8.17^{a}±0.1	8.30^{a}±0.3	8.19^{a}±0.2	8.4$^{a=}$±0.1	8.52$^{a=}$±0.2	0.040

*Means with different superscripts amongst treatments differed significantly (P<0.05) in a row.

T1= Control, T2= 0.15% Available Phosphorus with *Phytase* @ 1000 FTU/kg, T3= 0.18% Available Phosphorus with *Phytase* @ 1000 FTU/kg, T4= 0.21% Available Phosphorus with *Phytase* @ 1000 FTU/kg, T5= 0.15% Available Phosphorus with *Phytase* @ 2000 FTU/kg, T6= 0.18% Available Phosphorus with *Phytase* @ 2000 FTU/kg, T7= 0.21% Available Phosphorus with *Phytase* @ 2000 FTU/kg

***Bone mineralization*:** The super dosing of phytase in low-phosphorus diets significantly improved ($P < 0.05$) bone mineralization. The T7 group (0.21% available phosphorus with 2000 FTU/kg phytase) exhibited the highest tibia ash content (48.8%), tibia bone calcium (Ca) and phosphorus (P) deposition, at 17.9% and 8.52%, respectively. In contrast, the control group showed the lowest tibia ash content (42.5%), as well as tibia bone Ca (14.35%) and P (6.83%) deposition, compared to other low-phosphorus diets supplemented with phytase (Table 3.3).

DISCUSSION

In this study, FI during the 1st week was not affected by phytase super dosing in phosphorus-deficient diets. However, during the grower (days 8–21) and finisher (days 22–28) phases, phytase supplementation at 2000 FTU/kg in broiler feed containing 0.21% available phosphorus significantly impacted feed intake. These results align with the findings of Walters *et al.* (2019), who reported that phytase supplementation in low-phosphorus diets boosted broiler feed intake (FI) and body weight (BW) at doses of 2000–3000 FTU/kg. Similarly, Raut *et al*. (2018) observed that diets with 1000 FTU phytase showed the lowest feed consumption, though statistical analysis did not reveal significant differences. Furthermore, Manobhavan *et al*. (2016) reported that corn-soy-based diets with phytase super doses (at 2500 FTU/kg and 5000 FTU/kg) improved feed intake in low-phosphorus diets compared to a standard dose of 500 FTU/kg. Our results indicate that maximum weight gain was achieved by birds fed a low phosphorus diet containing 0.21% available phosphorus with phytase super dosing at 2000 FTU/kg. These findings agree with Broch *et al*. (2018), who found that phytase doses of 2051 FTU/kg and 2101 FTU/kg enhanced weight gain in broilers over 42 days. Babatunde *et al*. (2019) suggested that phytase efficacy in broilers depends on factors such as age, dose, and feeding duration, which influence both weight gain and phosphorus digestibility. Similarly, Walk and Rama Rao (2020) found that phytase supplementation at 449 FTU/kg or 2000 FTU/kg increased the broiler weight gain. Based on this data, it can be inferred that phytase super dosing with reduced mono-calcium phosphate (MCP) content in broiler diets improved phosphorus utilization, nutrient availability, and growth performance

This study also showed a significant weekly improvement in FCR, with the most efficient gains observed in T7 group (0.21% available phosphorus with phytase at 2000 FTU/kg). Consistently, Pieniazek *et al*. (2017) reported that FCR improved at a phytase dose of 2000 FTU/kg in diets deficient in available phosphorus. Hossain and Sajjad (2021) concluded that FCR was more efficient when phytase was supplemented at 1500 FTU/kg compared to diets containing 2500 FTU/kg. Martínez-Vallespín *et al*. (2022) further showed that broilers fed a low-phosphorus diet supplemented with phytase at 500, 1000, and 3000 FTU/kg from day 1–21 demonstrated better FCR as phytase dose increased.

Our study also revealed that phytase super dosing improved the digestibility of DM, CP, and EE, with the highest levels observed in T7 (0.21% available phosphorus with 2000 FTU/kg phytase). Walters *et al*. (2019) found similar results, reporting that a corn-soy-based diet with high phytase inclusion (2000 and 3000 FTU/kg) increased nutrient digestibility. Martínez-Vallespín *et al*. (2022) observed that broilers fed a low-phosphorus diet with increasing phytase inclusion at 1000 or 3000 FTU/kg showed higher apparent ileal digestibility of crude protein, phosphorus, and certain amino acids. Sampath *et al*. (2023) also reported that phytase inclusion above 500 FTU/kg improved nutrient utilization, consistent with the results observed in this study.

This study's results showed that tibia ash content (%), Ca (%), and P (%) were highest when a low phosphorus diet (0.21% available phosphorus) was supplemented with 2000 FTU/kg phytase. Leeson *et al*. (2000) similarly reported that diets with only 0.25% available phosphorus reduced tibia weight, tibia ash, and tibia P content, while phytase supplementation increased tibia weight and P content. Cozannet *et al*. (2023) found that phytase supplementation improved phosphorus digestibility and bone ash content, with available phosphorus increasing from 0.18% at 1000 FTU/kg to 0.20% at 2000 FTU/kg. Moradi *et al*. (2023) reported that bacterial 6-phytase at 500–1000 FTU/kg in broiler diets could replace 1.5 g/kg available phosphorus and 3 g/kg calcium without compromising body weight gain and growth performance. Cozannet *et al*. (2023) concluded that phytase (1000 or 2000 FTU/kg) improved phosphorus digestibility and bone ash content. Adding exogenous phytase to the diet enhances growth efficiency, nutrient digestibility, and mineral deposition. These effects are likely due to the hydrolysis of phytic acid in the diet, which increases the availability of various minerals and amino acids.

***Conclusion*:** Based on the research findings, it is concluded that reducing available phosphorus to 0.21% with the supplementation of phytase at 2000 FTU/kg improves feed intake (FI), feed conversion ratio (FCR), and body weight gain (BWG), enhancing overall growth performance. The digestibility percentages of dry matter (DM), crude protein (CP), and ether extract (EE) were highest in birds fed a diet with 0.21% phosphorus supplemented with phytase at 2000 FTU/kg. Super dosing phytase at 2000 FTU/kg effectively hydrolyzed the phytate bonds, improving phosphorus and calcium retention and promoting bone mineralization in the experimental birds.

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preparation, B.A., B.A., and W.A.; writing—review and editing, O.A.K., M.A., F.R., and W.A.

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REFERENCES

Babatunde, O.O., A.J. Cowieson, J.W. Wilson and O. Adeola. 2019. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. Poultry Science 98:2588-2597.

Baradaran, N., M.H. Shahir, H.R. Taheri and M.R. Bedford. 2021. Effect of sequential feeding of phosphorus-deficient diets and high-dose phytase on efficient phosphorus utilization in broiler chickens. Livestock Science 243:104368.

Boney, J.W. and J.S. Moritz. 2017. Phytase dose effects in practically formulated diets that vary in ingredient composition on feed manufacturing and broiler performance. Journal of Applied Poultry Research 26:273-285.

Broch, J., R.V. Nunes, C. Eyng, G.M. Pesti, C. de Souza, G.G. Sangalli, V. Fascina and L. Teixeira. 2018a. High levels of dietary phytase improves broiler performance. Animal Feed Science Technology 244:56-65.

Butani, J.B. and S. Parnerkar. 2015. Role of microbial phytase in broiler nutrition-a review. Journal of Livestock and Science 6:113-118

Cambra-López, M., V. Moset, M. del Carmen López, J. Sebastián Mesa, L. Carpintero, A. Donadeu, J. Dupuy, J. Macías-Vidal, A. Cerisuelo and P. Ferrer. 2021. Evaluation of phosphorus digestibility from monocalcium and dicalcium phosphate sources and comparison between total tract and prececal digestibility standard methods in broilers. Animals 11:3427.

Cozannet, P., M. Jlali, D. Moore, M. Archibeque and A. Preynat. 2023b. Evaluation of phytase dose effect on performance, bone mineralization, and prececal phosphorus digestibility in broilers fed diets with varying metabolizable energy, digestible amino acids, and available phosphorus concentration. Poultry Science 102:102755.

Dersjant-Li, Y., A. Awati, H. chulze and G. Partridge. 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. Journal of the Science of Food and Agriculture 95:878-896.

Gulizia, J.P., M.S. Rueda, F.K. Ovi, S.M. Bonilla, R. Prasad, M.E. Jackson, O. Gutierrez and W.J. Pacheco. 2022. Evaluate the effect of a commercial heat stable phytase on broiler performance, tibia ash, and mineral excretion from 1 to 49 days of age assessed using nutrient reduced diets. Journal of Applied Poultry Research 31:100276.

Horwitz, W. and G.W. Latimer. 2000. Association of official analytical chemists. Gaithersburg, MD, USA.

Hossain and Sajjad. 2021. Effects of phytase super dosing on performance, plasma mineral contents and bone mineralization in broiler chicken. (Doctoral dissertation, Chattogram Veterinary and Animal Sciences University Chattogram-4225, Bangladesh.

Humer, E., C. Schwarz and K. Schedle. 2015. Phytate in pig and poultry nutrition. Journal of Animal Physiology and Animal Nutrition (Berl) 99:605-625.

Iqbal, W., M.A. Yaseen, M.A. Rahman, S.A. Bhatti, M.S. Rahman, M.U. Yaqoob, F. Ahmad, M.U. Zahid and M. Shoaib. 2023. Effect of Phytase Supplementation on Growth Performance, Mineral Digestibility, and Tibia Calcium and Phosphorous in Broilers Fed Low Phosphorus Diets. Brazilian Journal of Poultry Science 25:eRBCA-2023.

Jiang, X.R., F.H. Luo, M.R. Qu, V. Bontempo, S.G. Wu, H.J. Zhang, H.Y. Yue and G.H. Qi. 2013. Effects of non-phytate phosphorus levels and phytase sources on growth performance, serum biochemical and tibia parameters of broiler chickens. Italian Journal of Animal Science 12:375-378.

Lalpanmawia, H., A.V. Elangovan, M. Sridhar, D. Shet, S. Ajith and D.T. Pal. 2014. Efficacy of phytase on growth performance, nutrient utilization and bone mineralization in broiler chicken. Animal Feed Science and Technology 192:81-89.

Leeson, S., H. Namkung, M. Cottrill and C.W. Forsberg. 2000. Efficacy of new bacterial phytase in poultry diets. Canadian Journal of Animal Science 80:527-528.

Lim, C.I., H.J. Choo and J.H. Park. 2024. Effect of phytase supplementation on performance, fecal excretion, and compost characteristics in broilers fed diets deficient in phosphorus and calcium. Journal of Animal Science and Technology 66:93-102

Manobhavan, M., A.V. Elangovan, M. Sridhar, D. Shet, S. Ajith, D.T. Pal and N.K.S. Gowda. 2016. Effect of super dosing of phytase on growth performance, ileal digestibility and bone characteristics in broilers fed corn–

soya-based diets. Journal of Animal Physiology and Animal Nutrition (Berl) 100:93-100.

Martínez-Vallespín, B., K. Männer, P. Ader and J. Zentek. 2022. Evaluation of high doses of phytase in a low-phosphorus diet in comparison to a phytate-free diet on performance, apparent ileal digestibility of nutrients, bone mineralization, intestinal morphology, and immune traits in 21-day-old broiler chickens. Animals 12:1955.

Moradi, S., M.R. Abdollahi, A. Moradi and L. Jamshidi. 2023. Effect of Bacterial Phytase on Growth Performance, Nutrient Utilization, and Bone Mineralization in Broilers Fed Pelleted Diets. Animals 13:1450.

Nuamah, E., U.M. Okon, E. Jeong, Y. Mun, I. Cheon, B. Chae, F.N.A. Odoi, D. Kim and N.-J. Choi. 2024. Unlocking Phytate with Phytase: A Meta-Analytic View of Meat-Type Chicken Muscle Growth and Bone Mineralization Potential. Animals 14:2090.

Pieniazek, J., K.A. Smith, M.P. Williams, M.K. Manangi, M. Vazquez-Anon, A. Solbak, M. Miller and J.T. Lee. 2017. Evaluation of increasing levels of a microbial phytase in phosphorus deficient broiler diets via live broiler performance, tibia bone ash, apparent metabolizable energy, and amino acid digestibility. Poultry Science 96:370-382.

Proszkowiec-Weglarz, M. and R. Angel. 2013. Calcium and phosphorus metabolism in broilers: Effect of homeostatic mechanism on calcium and phosphorus digestibility1 1Presented as a part of the Informal Nutrition Symposium “Metabolic Responses to Nutrition and Modifiers” at the Poultry Science Association’s annual meeting in Athens, Georgia, July 9, 2012. Journal of Applied Poultry Research 22:609-627.

Raut, S., M. Dhumal, M. Nikam and K. Khose. 2018. Influence of super dosing of phytase enzyme on performance of broiler chicken. International Journal of Livestock Research 8:220-231.

Sampath, V., S. Gao, J.H. Park and I.H. Kim. 2023. Exogenous phytase improves growth performance, nutrient retention, tibia mineralization, and breast meat quality in ross-308 broilers. Agriculture 13:1906.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and procedures of statistics: a biometrical approach.

Valable, A.-S., A. Narcy, M.J. Duclos, C. Pomar, G. Page, Z. Nasir, M. Magnin and M.P. Létourneau-Montminy. 2018. Effects of dietary calcium and phosphorus deficiency and subsequent recovery on broiler chicken growth performance and bone characteristics. Animal 12:1555-1563.

Valente Junior, D.T., J.L. Genova, S.W. Kim, A. Saraiva and G.C. Rocha. 2024. Carbohydrases and phytase in poultry and pig nutrition: a review beyond the nutrients and energy matrix. Animals 14:226.

Venter, K., W. Li, R. Angel, P.W. Plumstead, M. Proszkowiec-Weglarz, H. Enting and L.E. Ellestad. 2024. Calcium and phosphorus digestibility in broilers as affected by varying phytate concentrations from corn. Poultry Science 103:104191.

Veum, T.L. 2010. Phosphorus and Calcium Nutrition and Metabolism. Phosphorus and Calcium Utilization and Requirements in Farm Animals 94-111.

Walk, C.L. and S.V. Rama Rao. 2020. Dietary phytate has a greater anti-nutrient effect on feed conversion ratio compared to body weight gain and greater doses of phytase are required to alleviate this effect as evidenced by prediction equations on growth performance, bone ash and phytate degradation in broilers. Poulryt Science 99:246-255.

Walk, C.L. and S.V.R. Rao. 2020. Increasing dietary phytate has a significant anti-nutrient effect on apparent ileal amino acid digestibility and digestible amino acid intake requiring increasing doses of phytase as evidenced by prediction equations in broilers. Poultry Science 99:290-300.

Walters, H.G., M. Coelho, C.D. Coufal and J.T. Lee. 2019a. Effects of Increasing Phytase Inclusion Levels on Broiler Performance, Nutrient Digestibility, and Bone Mineralization in Low-Phosphorus Diets. Journal of Applied Poultry Research 28:1210-1225.

Woyengo, T.A. and C.M. Nyachoti. 2013. Anti-nutritional effects of phytic acid in diets for pigs and poultry–current knowledge and directions for future research. Canadian Journal of Animal Science 93:9-21.

Xia, C., Y. Liang, S. Bai, Y. He, A.U.R. Muhammad, H. Su and B. Cao. 2018. Effects of harvest time and added molasses on nutritional content, ensiling characteristics and in vitro degradation of whole crop wheat. Asian-Australasian Journal of Animal Sciences 31:354.